February 2026

QIAsymphony® PAXgene® Blood ccfDNA Kit Handbook

For purification of circulating cell-free DNA from plasma using the QIAsymphony SP instrument.

Important: To be used only in conjunction with plasma generated from PAXgene Blood ccfDNA Tubes.

For Research Use Only. Not for use in diagnostic procedures.

PreAnalytiX® GmbH

Garstligweg 8, 8634 Hombrechtikon, Switzerland

Produced by QIAGEN® GmbH for PreAnalytiX GmbH

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

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PreAnalytiX products are manufactured and distributed by QIAGEN® and BD for PreAnalytiX.

Contents

Kit Contents

QIAsymphony® PAXgene® Blood ccfDNA Kit	(192)
Catalog no.	768536
No. of preps	192
Proteinase K	5 × 10 mL
Reagent Cartridge*	2
Piercing Lid	2
Reuse Seal Set	2
Elution Microtubes CL, racked	2
Caps for Elution Microtubes†	2 × (55 × 8)
PAXgene Blood ccfDNA Purification Selection Tool*	1
Important Note	1

* Prefilled reagent cartridges include buffers that contain ethanol. See "Safety Information", page 9.

† Also available separately. See "Ordering Information", page 39.

Shipping and Storage

Reagent Storage and Handling

Store the QIAsymphony PAXgene Blood ccfDNA Kit upright at room temperature (15–25°C). The magnetic particles in the reagent cartridges (RCs) remain active when stored within this temperature range.

Note: The label on the QIAsymphony PAXgene Blood ccfDNA Kit box lists the expiration date of the kit. The expiration date is for the reagent cartridge.

Do not use the QIAsymphony PAXgene Blood ccfDNA Kit once it has expired.

The QIAsymphony PAXgene Blood ccfDNA Kit contains a ready-to-use Proteinase K solution that can be stored at room temperature (15–25°C).

Do not store RCs at temperatures below 15°C.

Opened QIAsymphony PAXgene Blood ccfDNA Kit RCs can be stored at room temperature (15–25°C) for up to 4 weeks, enabling cost-efficient reuse of reagents and more flexible sample processing, if properly resealed after use. If an RC is partially used, replace the cover of the trough containing the magnetic particles and seal the RC with the provided Reuse Seal Strips immediately after the end of the protocol run to avoid evaporation.

To avoid reagent evaporation, the RC should be open for a maximum of 15 h (including run times) at a maximum environmental temperature of 32°C. Incorrect storage of the kit components can lead to accelerated aging of buffers.

Running batches with low sample numbers (<24) will increase both the time that the RC is open and the required buffer volumes, potentially reducing the total number of sample preparations possible per cartridge.

Avoid exposure of the RCs to UV light (e.g., used for decontamination) as exposure may cause accelerated aging of the RCs and buffers.

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

Intended Use

The QIAsymphony PAXgene Blood ccfDNA Kit, to be used with the QIAGEN QIAsymphony SP instrument, is intended for automated isolation and purification of circulating cell-free DNA (ccfDNA) from plasma generated from human venous whole blood collected in the PAXgene Blood ccfDNA Tube.

The PAXgene Blood ccfDNA Tube is a plastic, closed, evacuated tube intended for the collection, anticoagulation, transportation, and storage of human whole blood specimens and the stabilization of ccfDNA. Using the tube in conjunction with the QIAsymphony PAXgene Blood ccfDNA Kit or the QIAGEN QIAamp® Circulating Nucleic Acid technology enables isolation of ccfDNA suitable for demanding downstream analytical assays.

The QIAsymphony PAXgene Blood ccfDNA Kit utilizes magnetic-particle technology for automated isolation and purification of ccfDNA from plasma, generated from specimens collected in PAXgene Blood ccfDNA Tubes.

The kit performance has been established in studies in which ccfDNA is purified from human plasma generated from specimens collected in PAXgene Blood ccfDNA Tubes. Users must validate the performance of the system in their laboratory for specific research applications.

For research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Warnings and Precautions

Safety Information

When working with chemicals and biological samples, always wear a suitable lab coat, disposable gloves, and protective goggles according to policies and procedures of your facility. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each PreAnalytiX kit and kit component.

- All chemicals and biological materials are potentially hazardous. Blood specimens and samples are potentially infectious and must be treated as biohazardous materials.
- Discard biohazardous materials and kit waste according to your local safety procedures.

Precautions

If buffers of the kit are spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water and then with 1% (v/v) sodium hypochlorite (bleach).

The following hazard and precautionary statements apply to components of the QIAsymphony PAXgene Blood ccfDNA Kit.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

MBS3	Contains: Sodium azide. Warning! May be harmful if swallowed. Wear protective gloves/protective clothing/eye protection/face protection.
Proteinase K 	Contains: Proteinase K. Danger! May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. If exposed or concerned: Call a poison center or doctor/physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing.

QSE2 	Danger! Causes severe skin burns and eye damage. Dispose of contents/container to an approved waste disposal plant. **If in eyes:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **If on skin (or hair):** Remove/take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a poison center or doctor/physician. Store locked up. Wear protective gloves/protective clothing/eye protection/face protection.
QSB9 	Contains: Ethanol. Danger! Highly flammable liquid and vapor. Causes serious eye irritation. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Wear protective gloves/protective clothing/eye protection/face protection.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsymphony PAXgene Blood ccfDNA Kit is tested against predetermined specifications to ensure consistent product quality.

Description and Principle

Summary and Explanation

Circulating cell-free DNA (ccfDNA) is present in plasma usually as short fragments (<1000 bp). The concentration of ccfDNA in plasma is usually low (can range from 1 to 100 ng/mL) and varies considerably between individuals. The PAXgene Blood ccfDNA Tube in combination with the QIAsymphony PAXgene Blood ccfDNA Kit constitutes a standardized workflow for collection, storage, and transport of blood; the stabilization of DNA in a closed tube; and the subsequent ccfDNA isolation and purification from human plasma using the QIAsymphony SP instrument.

Protocols on the QIAsymphony SP instrument are provided for extraction of ccfDNA from 2.4 and 4.8 mL plasma generated from PAXgene Blood ccfDNA Tubes by double centrifugation. Plasma is transferred and processed in a secondary tube on the QIAsymphony SP instrument.

Alternatively, primary tube handling protocols on the QIAsymphony SP instrument are available for 2.4 and 4.8 mL plasma input volume as customized protocols*. In this case, there is no need for a second centrifugation step or plasma transfer into a secondary tube. The primary blood collection tube can be directly processed on the QIAsymphony SP instrument.

*Modified QIAGEN protocols or custom protocols for QIAsymphony SP developed to meet your specific requirements can be requested and purchased from QIAGEN. Please contact QIAGEN Technical Services or your local distributor.

Specimen Collection and Preparation

The purification procedure is optimized for use with plasma generated from blood collected in PAXgene Blood ccfDNA Tubes (cat. no. 768115). For information on blood collection, handling of these tubes, and plasma preparation, see the *PAXgene Blood ccfDNA Tube Instructions for Use* which can be found under the Product Resource tabs and Resources tabs of the product pages on **www.qiagen.com** and **www.preanalytix.com**, respectively.

Plasma preparation can be done using **(A)** the standard double-centrifugation protocols or **(B)** via customized primary tube handling protocols: direct processing of the one-time centrifuged PAXgene Blood ccfDNA Tubes on the QIAsymphony SP instrument.

Plasma can be stored **(C)** frozen and can be prepared after thawing of plasma samples.

A) Plasma preparation from blood for standard protocols

1. Gently invert the PAXgene Blood ccfDNA Tube 8 times prior to centrifugation.
   Note: For best performance for samples stored or transported refrigerated prior to centrifugation, blood samples should be incubated for 30 min at 30–37°C or at least allowed to return to room temperature before processing.
2. Centrifuge the PAXgene Blood ccfDNA Tube at 15–25°C for 15 min at 1600–3000 × *g* (first centrifugation) using a balanced swing out bucket centrifuge. If braking is preferred, it is recommended to use medium level braking, and it should be validated for your specific workflow.
3. Pipette the plasma into a 15 mL conical bottom centrifuge tube (not provided), making sure to not disturb the nucleated cellular fraction.
4. Centrifuge the 15 mL conical bottom centrifuge tube at 15–25°C for 10 min at 1600–3000 × *g* (second centrifugation) using a balanced centrifuge.

Note: Do not exceed the secondary tube manufacturer's maximum recommended centrifugation speed.

5. Pipette the required plasma volume (see section "Sample volume", page 24) into a 14 mL, 17 × 100 mm polystyrene, round-bottom tube, making sure to not disturb the residual blood cell pellet, if present.
6. Transfer the round-bottom tube with the plasma sample into the tube carrier and load the tube carrier in the sample input drawer of the QIAsymphony SP instrument.

   Note: For maximum ccfDNA yield, process the maximum volume of plasma available.

   Note: Prevent formation of foam in or on the surface of plasma samples during pipetting. Foam or air bubbles on samples can lead to pipetting of wrong sample volume.

   Note: After transfer of plasma in secondary tube ccfDNA is stable in plasma at 15–25°C for up to 3 days or at 2–8°C for up to 7 days. For longer storage, we recommend freezing aliquots at –20°C or –80°C.

   Note: When using previously stored plasma samples (e.g., stored at 2–8°C or frozen at –20°C or –80°C), these should be equilibrated to room temperature (15–25°C) before starting the run; for frozen samples, please see section "C) Freezing and thawing of plasma samples processed from the PAXgene Blood ccfDNA Tube".

B) Plasma preparation from blood for customized primary tube* handling on the QIAsymphony SP instrument

* Primary tube handling protocols are not part of the regular protocols for the QIAsymphony PAXgene Blood ccfDNA Kit can are available as customized protocols. Modified QIAGEN protocols or custom protocols developed to meet your specific requirements can be requested and purchased from QIAGEN. Please contact QIAGEN Technical Services or your local distributor.

1. Gently invert the PAXgene Blood ccfDNA Tube 8 times prior to centrifugation.
   Note: For best performance for samples stored or transported refrigerated prior to centrifugation, blood samples should be incubated for 30 min at 30–37°C or at least allowed to return to room temperature before processing.
2. Centrifuge the PAXgene Blood ccfDNA Tube at 15–25°C for 15 min at 3000 × *g* using a balanced swing out bucket centrifuge. If braking is preferred, it is recommended to use medium level braking and it should be validated for your specific workflow.
3. Quantify the plasma volume in each tube after removal from the centrifuge bucket with the PAXgene Blood ccfDNA Purification Protocol Selection Tool provided as a kit content (Figure 1). Upon removal of the tube from the centrifuge, the teal arrow on the tool is aligned with the plasma/cell interface. The blue lines indicate if the plasma level is sufficient for the 2.4 mL or 4.0 mL primary tube handling protocol. A minimum plasma column height of 2.3 cm is needed for the 2.4 mL protocol and a minimum of 3.4 cm is needed for the 4.0 mL protocol.

   In case a clear separation of plasma and cell fraction did not occur, or phases were accidentally mixed upon removal from the centrifuge, centrifugation should be repeated.

   Note: Check for a clear separation before placing the tube on the instrument.

Figure 1. PAXgene QIAsymphony Blood ccfDNA Purification Protocol Selection Tool. The plasma volume in each tube is quantified after removal from the centrifuge bucket with the PAXgene Blood ccfDNA Purification Protocol Selection Tool provided as a QIAsymphony PAXgene Blood ccfDNA Kit (CE-IVD) content. Upon removal of the tube from the centrifuge, the teal arrow on the tool is aligned with the plasma/cell interface. The blue lines indicate if the plasma level is sufficient for the 2.4 or 4.0 mL primary tube handling protocol.

4. Remove the Hemogard Closure Cap from the PAXgene Blood ccfDNA Tubes before placing it on the QIAsymphony SP instrument for direct ccfDNA extraction.

   Note: Removing tube closure and handling of open tubes should be done carefully to reduce potential risk of sample spillage, cross contamination between tubes and blood exposure.

5. Place the opened PAXgene Blood ccfDNA Tubes that contain sufficient plasma into the tube carrier and load the tube carrier in the sample input drawer of the QIAsymphony SP instrument.

C) Freezing and thawing of plasma samples processed from the PAXgene Blood ccfDNA Tube

1. For freezing, transfer the plasma into suitable tubes (e.g., cryovials) placed in an appropriate rack.
2. For long-term storage, aliquot and freeze plasma at –20°C or –80°C in cryogenic tubes. ccfDNA is stable in frozen plasma for up to 5 years. For a current listing of the performance characteristics for the PAXgene Blood ccfDNA Tube including ccfDNA stability in frozen plasma, visit **www.PreAnalytiX.com**.
3. Thaw aliquot tubes for 30 min at 30–37°C to minimize formation of cryoprecipitates.

   Note: Do not thaw at low temperatures (e.g., 4°C), which may induce formation of cryoprecipitates.
4. If cryoprecipitates remain in the plasma, vortex the tube for 30 s after thawing and use the sample for the QIAsymphony SP instrument ccfDNA isolation procedure without further treatment.

   Note: It is not recommended to centrifuge the plasma to remove cryoprecipitates because they may contain ccfDNA.

Principles of the Procedure

The QIAsymphony SP instrument technology combines the speed and efficiency of anion-exchange–based nucleic acid purification with the convenient handling of magnetic particles (Figure 3, page 20). The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples and consists of 3 steps: bind, wash, and elute. Users can choose between different sample input volumes.

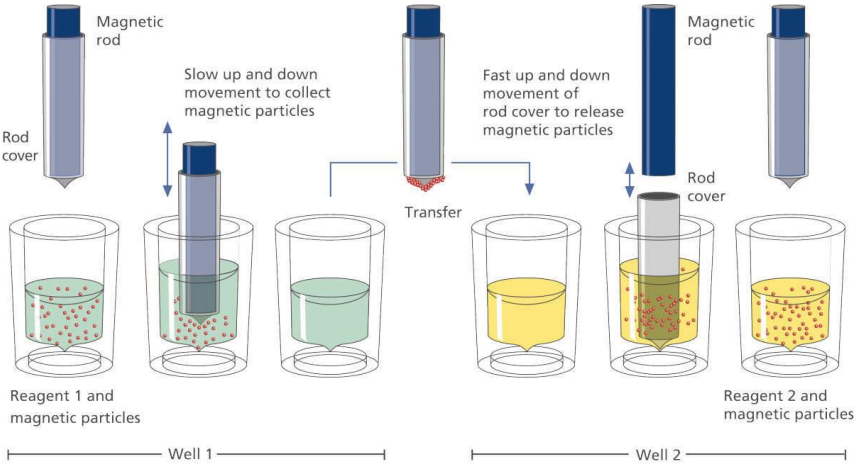

Figure 2. Schematic of the QIAsymphony SP principle. The QIAsymphony SP processes a sample containing magnetic particles as follows. A magnetic rod protected by a rod cover enters a well containing the sample and attracts the magnetic particles. The magnetic rod cover is positioned above another well and the magnetic particles are released. The QIAsymphony SP uses a magnetic head containing an array of 24 magnetic rods, and can therefore process up to 24 samples simultaneously. Steps 1 and 2 are repeated several times during sample processing.

The QIAsymphony PAXgene Blood ccfDNA Kit magnetic-particle technology enables purification of high-quality ccfDNA that is free of proteins, nucleases, and other impurities. The QIAsymphony SP instrument performs all steps of the purification procedure. Up to 96 samples, in batches of 24, are processed in a single run. For isolation of genomic DNA (gDNA) from the nucleated cellular fraction of blood collected into PAXgene Blood ccfDNA Tubes on the QIAsymphony SP instrument, refer to the instructions provided in the *PAXgene Blood ccfDNA Tube Instructions for Use* (**www.preanalytix.com**).

During the first processing step, plasma proteins are digested by Proteinase K while the ccfDNA binds to the surface of magnetic particles. Depending on the chosen protocol line (Figure 3) predominately small ccfDNA fragments or small and large

ccfDNA fragments are isolated. Three wash steps guarantee that contaminants are removed. Finally, ccfDNA is diluted from the magnetic particles and is ready for use in downstream applications.

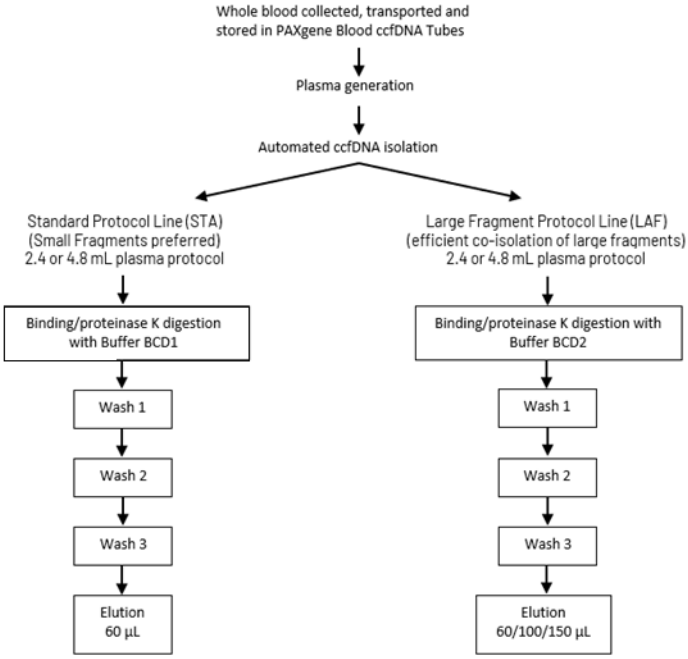

Figure 3. Standard and large fragment protocol lines. With the standard protocols (STA) predominately small ccfDNA fragments are isolated while large fragment protocols (LAF) efficiently co-purify large and small ccfDNA fragments. Both protocol lines consist of one protocol for 2.4 mL and one protocol for 4.8 mL plasma input.

Equipment and Reagents to be Supplied by User

Consumables

- 8-Rod Covers (cat. no. 997004)
- Sample Prep Cartridges, 8-well (cat. no. 997002)
- Filter-Tips, 200 µL (cat. no. 990332), and 1500 µL (cat. no. 997024)
- Sterile, aerosol-barrier, RNase-free pipette tips
- 14 mL Falcon® polystyrene round-bottom tube, 17 × 100 mm (Corning, 352051)
- PAXgene Blood ccfDNA Tubes (cat. no. 768115)
- Tip Disposal Bags (cat. no. 9013395)

Equipment

- QIAsymphony SP* (cat. no. 9001297)
- Optional: QIAsymphony Cabinet SP* (cat. no. 9020244)
- Pipettes* (200 µL to 5 mL)
- Centrifuge* capable of attaining at least 1900 × *g* and equipped with a swing-out (optional) rotor and buckets to hold PAXgene Blood ccfDNA Tubes
- Vortexer

* Ensure that instruments have been checked and calibrated regularly according to the manufacturer's recommendations.

Elution into Elution Microtubes CL (provided with the kit)

Equipment

- Spatula to remove the lower plate from the elution microtube rack
- Cooling Adapter, EMT, v2, Qsym

Elution into 1.5 mL Eppendorf® DNA LoBind Tube or 1.5 mL Sarstedt® tubes (not provided)

Consumables

- 1.5 mL DNA LoBind Tube, Snap Cap (Eppendorf, cat. no. 0030108.051)
- Micro tube 1.5 mL, PP, NON-SKIRTED (Sarstedt, cat. no. 72.607) with Screw cap, neutral (Sarstedt, cat. no. 65.716.725)

Equipment

- Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym
- Cooling Adapter, 2 mL, v2, Qsym

For other possible elution formats, contact QIAGEN Technical Services or your local sales representative for a customized protocol.

Automated Purification on the QIAsymphony SP

The QIAsymphony SP instrument makes automated sample preparation easy and convenient. Samples, reagents and consumables, and eluates are separated in different drawers. Simply load samples, reagents provided in special cartridges, and pre-racked consumables in the appropriate drawer before run. Start the protocol and remove purified ccfDNA from the "Eluate" drawer after sample processing is completed. Refer to the user manuals supplied with your instrument for operating instructions.

Even though the QIAsymphony SP was designed as a benchtop instrument, the use of the QIAsymphony Cabinet SP will enhance the convenience of using this robotic system. The QIAsymphony Cabinet SP is specifically designed for correct positioning of the QIAsymphony SP instrument. The QIAsymphony Cabinet SP contains a waste compartment, into which used tips from the worktable can be rejected.

The complete PAXgene Blood ccfDNA workflow is described in "Protocol: Purification of ccfDNA from Human Plasma Generated from Human Whole Blood Collected into PAXgene Blood ccfDNA Tubes", page 32.

"Sample" drawer

Samples are loaded via the tube carriers into the "Sample" drawer. Plasma has to be filled into secondary sample tubes and specific labware files have to be selected on the user interface of the QIAsymphony SP (specified in Table 1 and Table 2).

Additionally, Proteinase K is loaded via the sample tube carrier in slot A, because this enzyme is not part of the Reagent Cartridges but is provided in glass vials with the kit.

The amounts of Proteinase K that has to be placed on the instrument is shown in Table 3.

Table 1. Sample drawer setup for purification of ccfDNA from plasma

Sample type	Human plasma generated from blood collected in PAXgene ccfDNA Tubes
Sample input volume	2.4 mL* (PAXcircDNA_STA_2400 or PAXcircDNA_LAF_2400) or 4.8 mL* (PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800)
Secondary sample tubes	14 mL, 17 × 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051)
Inserts	n/a
Other	Proteinase K required in 14 mL, 17 × 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051); only use positions 1 and 2 of the tube carrier (for slot A)

* For detailed information see "Sample volume", page 24.
n/a = not applicable.

Sample volume

To transfer the complete 2.4 mL (PAXcircDNA_STA_2400 or FT_PAXcircDNA_LAF_2400) and 4.8 mL (PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800) sample during the isolation procedure, the QIAsymphony SP requires an additional 0.4 mL (2.8 mL total) and 0.5 mL (5.3 mL total) of sample, respectively, to account for the void volume that is not transferred to the isolation procedure. If less volume is provided, samples are flagged as "unclear" but are still processed. If less than 1800 µL sample are provided for the PAXcircDNA_STA_2400 or PAXcircDNA_LAF_2400 protocol, or less than 4200 µL for the PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800 protocol, samples are flagged as "invalid" and are not processed.

Important: With the sample tube format "BD_FIX #352051 FalconPP 17 × 100," the QIAsymphony does not perform liquid level detection before aspiration of the sample. In this case, a void volume of 0.1 mL is required to make sure that 2.4 mL sample for the PAXcircDNA_STA_2400 or PAXcircDNA_LAF_2400 protocols and 4.8 mL for the PAXcircDNA_STA_4800 or PAXcircDNA_LAF_4800 protocols are

transferred. Less than 2.5 mL or 4.9 mL, respectively, may cause foam to form during sample processing.

Table 2. Sample tubes for tube carrier

Name on touchscreen	Supplier (cat. no.)	Material	Sample volume (mL)	
			PAXcircDNA STA 2400 PAXcircDNA LAF 2400	PAXcircDNA STA 4800 PAXcircDNA LAF 4800
BD #352051 FalconPP 17 × 100	Corning* (352051)	14 mL Falcon polystyrene round-bottom rube 17 × 100 mm	2.8 † 1.8 †§	5.3 † 4.2 †§
BD_FIX #352051 FalconPP 17× 100	Corning* (352051)	14 mL Falcon polystyrene round-bottom tube 17 × 100 mm	2.5 ‡¶	4.9 ‡¶

* Previously supplied by BD™.

† Minimum sample volume required per sample per protocol (including void volume); clot detection possible.

‡ Minimum sample volume required per sample per protocol (including void volume); clot detection not possible.

§ Reduced minimum sample volume. Make sure to use the "enable less sample" mode, which uses all available liquid in combination with liquid level detection and clot detection. The "enable less sample" mode results in "unclear" flagging of samples.

¶ Reduced minimum sample volume (mL) to minimize dead volumes. FIX labware is designed for this purpose and does not support liquid level detection or clot detection. FIX sample tubes impose aspiration restriction. Sample is aspirated at a defined height in the tube. This height is specified by the volume of sample to be transferred. Therefore, it is essential to make sure to use the volume listed in the table.

Table 3. Preparation of proteinase K in position 1 (and if required, in position 2) of slot A

Sample number	PAXcircDNA 2400*		PAXcircDNA 4800*	
	STA (µL)	LAF (µL)	STA (µL)	LAF (µL)
8	1980	2060	2860	3020
24	3740	3980	6380	6860
96	11,660	11,660‡ (tube 1) & 2060 (tube 2)	11,660†	11,660‡ (tube 1) & 11,660 (tube 2) & 3020 (tube 3)

* For each sample, 110 µL for PAXcircDNA STA 2400 or 220 µL for PAXcircDNA STA 4800, are required, plus an additional void volume of 1100 µL [(n × 110 or 220 µL) + 1100 µL].

† For each sample, 120 µL for PAXcircDNA LAF 2400 or 240 µL for PAXcircDNA LAF 4800, are required, plus an additional void volume of 1100 µL [(n × 120 or 240 µL) + 1100 µL].

‡ If more than 11,660 µL are required, use additional tubes (Corning, cat. no. 352051). An additional void volume of 1100 µL is required for each additional tube used.

Note: Tubes containing Proteinase K are placed in a tube carrier. The tube carrier containing the Proteinase K must be placed in positions 1 and 2 in slot A of the "Sample" drawer. We recommend using 14 mL, 17 × 100 mm polystyrene, round-bottom tubes (Corning, cat. no. 352051) for Proteinase K.

Reagent cartridges

Reagents for purification of ccfDNA from human plasma generated from whole blood collected in PAXgene Blood ccfDNA Tubes are contained in an innovative RC (Figure 4). Only Proteinase K has to be loaded in 14 mL Falcon tubes on slot A via a tube carrier (see "Sample" drawer, page 23). Each trough of the RC contains a particular reagent, such as magnetic particles, binding buffers, wash buffers, or elution buffers. Partially used RC can be reclosed with Reuse Seal Strips for later use. This avoids generating waste due to leftover reagents at the end of the purification procedure.

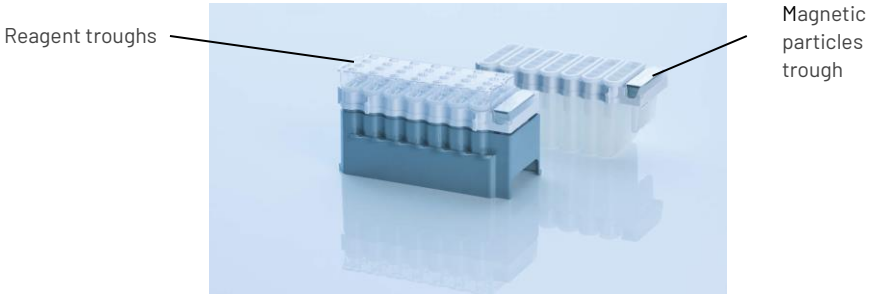

Figure 4. Reagent cartridge (RC). The reagent cartridge contains all reagents required for the protocol run.

Before using a RC for the first time, remove the seal from the trough containing the magnetic particles and replace it with the trough cover. Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 min before first use.

Note: Magnetic particles can change color. This has no influence on performance.

Place the RC into the reagent cartridge holder. Place the piercing lid on top of the reagent cartridge.

Note: The piercing lid is sharp; use caution when placing it onto the RC. Make sure to place the piercing lid onto the RC in the correct orientation.

After the magnetic-particle trough cover is removed, the RC is subsequently loaded into the "Reagents and Consumables" drawer.

Partially used RCs can be stored until needed again (see "Reagent Storage and Handling" on page 6).

Note: Proteinase K must be added (see "Things to do before starting" on page 32).

Note: Make sure that RCs, magnetic-particle troughs and Proteinase K bottles are not interchanged between different kit lots.

Loading plasticware

Sample prep cartridges, 8-Rod Covers (both pre-racked in unit boxes), and disposable filter-tips (200 µL tips in blue racks, 1500 µL tips in gray racks; not provided) are loaded into the "Reagents and Consumables" drawer (see Table 4, Table 7, and Table 8 for the consumables required).

For plasticware ordering information, see page 39.

Note: Both types of tips have filters to prevent cross-contamination.

Tip rack slots on the QIAsymphony worktable can be filled with either type of tip rack. The QIAsymphony SP will automatically identify the type of tips loaded during the inventory scan.

Note: Do not refill tip racks before starting another protocol run. The QIAsymphony SP can use partially used tip racks.

Table 4. Reagent and Consumables drawer setup for purification of ccfDNA

Drawer Setup	Reagent and Consumables
Position A1 and/or A2	**Reagent cartridge**
Position B1	n/a
Tip rack holder 1–17	Disposable filter-tips, 200 µL or 1500 µL
Unit box holder 1–4	Unit boxes containing sample prep cartridges or 8-Rod Covers
Position A1 and/or A2	Reagent cartridge

n/a = not applicable.

"Waste" drawer

Sample prep cartridges and 8-Rod Covers used during a run are pre-racked in empty unit boxes in the "Waste" drawer (Table 5). Make sure that the "Waste" drawer contains sufficient empty unit boxes for plastic waste generated during the protocol run.

Note: Ensure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If you are using 8-Rod Cover boxes for collecting used sample prep cartridges and 8-Rod Covers, ensure that the box spacer has been removed.

A bag for used filter-tips must be attached to the front side of the "Waste" drawer. If a QIAsymphony Cabinet SP is used, the waste compartment, into which used tips from the worktable are ejected, must be checked for sufficient space to collect waste tips.

Note: The presence of a tip disposal bag is not checked by the system. Make sure that the tip disposal bag is properly attached before starting a protocol run. For more information, see the user manuals provided with your instrument.

A waste container collects all liquid waste generated during the purification procedure. The "Waste" drawer can only be closed if the waste container is in place. Furthermore, a liquid level sensor detects the level of liquid in the waste container.

The system notifies the user if there is not enough capacity left in the container for liquid waste from the queued batch.

Table 5. Waste drawer setup for purification of ccfDNA from plasma

Waste drawer position	Consumable or insert item
Unit box holder 1–4	**Empty unit boxes**
Waste bag holder	Tip Disposal Bags
Liquid waste bottle holder	Empty liquid waste bottle

"Eluate" drawer

The required elution format is loaded into the "Eluate" drawer (see Table 6). Do not load a 96 well plate into "Elution slot 4". Use "Elution slot 1" with the corresponding cooling adaptor so that eluates will be cooled at the end of the run.

Table 6. Eluate drawer setup for purification of ccfDNA from plasma

Supplier	Material	Category	Name on touchscreen	Adapter on Elution slot 1 (cooled)
QIAGEN (supplied with kit; 19588)	Elution Microtube CL 96	Deep Well	QIA#19588*EMTR	Cooling Adapter, EMT, v2, Qsym
Eppendorf (0030108.051)	1.5 mL DNA LoBind Tube	Tube,1.5 mL	EP#0030108.051†T1.5 Snap Cap	Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym
Sarstedt (72.607)	1.5 mL Micro tube, PP, NON-SKIRTED	Tube, 1.5 mL / Tube, 1.5 mL Adapter V1	Sarstedt (72.607)	1.5 mL Micro tube, PP, NON-SKIRTED

* Indicates labware that can be cooled using a cooling adapter with bar code (transferable and usable on QIAsymphony AS).

†Indicates labware that can be cooled using a cooling adapter without bar code (non-transferable and not usable on QIAsymphony AS).

Inventory scan

Before starting a run, the instrument checks that sufficient consumables for the queued batch(es) have been loaded into the corresponding drawers (Table 7 and Table 8).

Table 7. Consumables required for purification of ccfDNA from plasma (PAXcircDNA 2400 STA and PAXcircDNA 2400 LAF)

Number of samples	24	48	96
Reagent cartridges*	1	1	2
Sample prep cartridges*	15	30	60
8-Rod Covers†	3	6	12
1500 µL tips‡	64	128	256
200 µL tips‡	24	48	96

* There are 28 sample prep cartridges/unit box.

† There are twelve 8-Rod Covers/unit box.

‡ There are 32 tips/tip rack; depending on the reagent cartridge status, the inventory scan requires additional tips (two 200 µL and nine 1500 µL tips).

Table 8. Consumables required for purification of ccfDNA from plasma (PAXcircDNA 4800 STA and PAXcircDNA 4800 LAF)

Number of samples	24	48	96
Reagent cartridges*	1	1	2
Sample prep cartridges*	18	36	72
8-Rod Covers†	3	6	12
1500 µL tips‡	104	200	392
200 µL tips‡	24	48	96

* There are 28 sample prep cartridges/unit box.

† There are twelve 8-Rod Covers/unit box.

‡ There are 32 tips/tip rack; depending on the reagent cartridge status, the inventory scan requires additional tips (two 200 µL and nine 1500 µL tips).

Protocol: Purification of ccfDNA from Human Plasma Generated from Human Whole Blood Collected into PAXgene Blood ccfDNA Tubes

Important points before starting

- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.
- Blood must be collected in PAXgene Blood ccfDNA Tubes (cat. no. 768115). For handling of these tubes and blood collection instructions see the PAXgene Blood ccfDNA Tube Product Circular provided with the PAXgene Blood ccfDNA Tubes.
- All steps of the QIAsymphony PAXgene Blood ccfDNA protocol for purification of ccfDNA from plasma should be performed at 15–25°C.

Things to do before starting

- Blood samples filled in PAXgene Blood ccfDNA Tubes are stable at room temperature (15–25°C) for up to 10 days or at higher temperatures (up to 37°C) for up to 3 days until centrifugation and plasma processing.
  Note: For information on blood collection and stability see the *PAXgene Blood ccfDNA Tube Instructions for Use*, which can be found under the Product Resource tabs and Resources tabs of the product pages on **www.qiagen.com** and **www.preanalytix.com**, respectively.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the sealed or covered trough containing the magnetic particles vigorously for at least 3 min before first use.

Preparation of sample material

For plasma preparation from blood collected in PAXgene Blood ccfDNA Tubes, see "Specimen Collection and Preparation" on pages 14-18.

Automated ccfDNA isolation on the QIAsymphony SP

1. Ensure that the QIAsymphony SP is switched on.

   The power switch is located at the bottom left corner of the QIAsymphony SP.
2. Ensure that the "Waste" drawer is prepared properly and perform an inventory scan of the "Waste" drawer, including the tip chute and liquid waste. Replace the tip disposal bag if necessary.
3. Load the required reagent cartridge and consumables (see Table 3 on page 26, Table 4 on page 29, and Table 7 on page 31) into the "Sample" and "Reagents and Consumables" drawers and perform an inventory scan of the "Reagents and Consumables" drawer.
4. Load the required elution format into the "Eluate" drawer.

   Place the adapter required for the specific elution format onto the selected elution position. If Elution Microtubes CL are used in the eluate cooling position ("Elution Slot 1"), remove the lower plate from the elution microtube rack using a spatula. Do not load a 96-well plate onto "Elution slot 4".
5. Load the samples (from section "Preparation of sample material", step 4 on page 23) into the "Sample" drawer.
6. Using the touchscreen, enter the required information for each batch of samples to be processed.

   Enter the following information:

   - Sample information (change default tube format; choose the "Select all" button on the sample view screen, and select "BD #352051 FalconPP

17 × 100" or "BD_FIX #352051 FalconPP 17 × 100" from the "Tube Insert 00" sheet)

- Protocol ("Assay Control Set") to be run. Choose the best suited protocol variant as described in Figure 3, page 20.
- Output position and if required elution volume.

After information about the batch has been entered, the status changes from "LOADED" to "QUEUED". As soon as one batch is queued the **Run** button appears.

7. Press **Run** to start processing.

   All processing steps are fully automated. The time elapsed is displayed.

   At the end of the protocol run, the status of the batch changes from "RUNNING" to "COMPLETED".

   It is recommended to use the "Elution slot 1" because this slot is able to cool the eluates after the run is complete.

8. After the QIAsymphony protocol finishes, remove the elution format containing the purified ccfDNA and seal with the appropriate caps.

   If the "Eluate" drawer is opened and not reclosed when a batch is running (e.g., if elution racks that contain eluates are removed), the run will be paused and an inventory scan of the "Eluate" drawer will be performed. A message window appears during the scan and must be closed (by pressing **Close**) before the run can be restarted.

   Result files are generated for each elution plate.

9. If the Microtubes CL were used, put the bottom plate back onto the rack for storage.

10. Store the purified ccfDNA at –15°C to –30°C or at –65°C to –90°C.

    In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. If magnetic particles need to be removed before performing downstream application, tubes or plates containing eluates should be spun down,

and eluates should be transferred to a clean tube. Alternatively, a suitable magnet can be used.

11. If the reagent cartridge is only partially used, seal it with the Reuse Seal Strips (provided) and close the enzyme tubes with screw caps immediately after the end of the protocol run to avoid evaporation. Remove the enzyme rack and store it at 2–8°C.
12. Discard used sample tubes and waste according to your local safety regulations.

    See "Safety Information", page 9.
13. Clean the QIAsymphony SP.

    Follow the maintenance instructions in the user manuals supplied with your instrument. Make sure to clean the tip guards regularly to minimize the risk of cross-contamination.
14. Close the workstation drawers and switch off the QIAsymphony SP.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving problems that may arise. For contact information and a list of Frequently Asked Questions visit the respective product page on **www.preanalytix.com** or **www.qiagen.com**.

	Comments and suggestions
General handling	
a) Overnight runs	To optimize throughput, the PAXgene Blood ccfDNA Kit supports processing samples overnight, since eluates are cooled on the QIAsymphony SP instrument at the end of a run. During long cooling periods, eluate volumes can differ slightly from volumes directly after the run, depending on temperature and humidity in the laboratory. For example, if the chosen volume was 60 µL at 15–25°C and 30–60% humidity, the volume can be in the range of 50–85 µL after 12 h storage on the instrument.
b) Error message displayed in the touchscreen	If an error message is displayed during a protocol run, refer to "Troubleshooting" in the *QIAsymphony SP User Manual*.
c) Instrument issue during the run	If the run stops because of an instrument issue during a run, the samples can be rescued via specific sample rescue protocols combined with a modified manual ccfDNA isolation procedure based on the QIAamp Circulating Nucleic Acid Kit. For sample rescue-protocols, please contact Technical Services at **www.preanalytix.com** or call your local distributor.

	Comments and suggestions
Cryoprecipitates occur after thawing of plasma samples	
a) Cryoprecipitates form in the plasma	To avoid formation of cryoprecipitates, the plasma can be thawed at 30°C for 30 min instead of room temperature. Do not thaw at low temperatures (e.g., 4°C). Vortex the tube for 30 s after thawing and use the sample for the QIAsymphony SP ccfDNA isolation procedure.
b) Low ccfDNA yield from plasma after removing cryoprecipitates	Do not centrifuge the plasma to remove cryoprecipitates, as they may contain ccfDNA.
Insufficient plasma from PAXgene Blood ccfDNA Tubes	
a) Less than 10 mL blood collected in PAXgene Blood ccfDNA Tube	Ensure that 10 mL blood is collected in the PAXgene Blood ccfDNA Tube (see the PAXgene Blood ccfDNA Tube Product Circular).
b) Hematocrit above specification	The QIAsymphony PAXgene Blood ccfDNA Kit is intended for purification of cell-fee circulating DNA from human whole blood with hematocrit of less than 51% (male) or less than 47% (female).
c) Low plasma yield after centrifugation	Longer storage or transport times can have an impact on plasma yields.
Stabilization of ccfDNA is impacted	
a) Compromised relative amount of ccfDNA	Optimal temperature range for transport and storage of blood collected in PAXgene Blood ccfDNA Tubes is 15–25°C. Longer incubation at high (e.g., >37°C) or low (e.g., <4°C) temperatures can have a negative impact on the relative amount of ccfDNA. Carryover of white blood cells during plasma generation will lead to genomic DNA contamination in the eluates of the ccfDNA isolation procedure and dilution of the original ccfDNA. Therefore careful transfer of plasma without disturbing the buffy coat after centrifugation of the blood is essential. To avoid even minimal cell carryover, a second centrifugation step as described on page step 2 could be helpful.

		Comments and suggestions
ccfDNA does not perform well in downstream applications		
a)	Eluate concentrated by vacuum centrifugation	Do not concentrate the eluate by vacuum centrifugation (e.g., in a SpeedVac® or similar instrument). This can lead to degradation due to high temperatures and concentrated salts in the eluate, which can interfere with downstream applications.
b)	Insufficient amount of ccfDNA used in downstream application	Quantify the purified ccfDNA by suitable methods like capillary electrophoresis (e.g., QIAGEN QIAxcel®, Agilent® BioAnalyzer) or sensitive assays (QIAGEN QIAxpert®, Qubit®, qPCR).
c)	Bead carryover	In general, magnetic particles are not carried over into eluates. If carryover does occur, magnetic particles in eluates will not affect most downstream applications. In case very high portions of eluates are needed for specific downstream assays, eluates could be spun down and transferred to a clean tube.
Low ccfDNA yield		
a)	Less than 10 mL blood collected into the PAXgene Blood ccfDNA Tube	Ensure that 10 mL blood is collected into the PAXgene Blood ccfDNA Tube (see the *PAXgene Blood ccfDNA Tube Instructions For Use*). Together with 1.5 mL of cell stabilization additive a tube completely filled should contain a total volume of blood and additive of 11.5 mL.
b)	Insufficient plasma processed by the instrument	Ensure that sufficient amount of plasma is loaded on the instrument. The void volumes needed depend on the chosen protocol and the sample input format used (see Table 1 on page 24 and Table 2 on page 25)
c)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
Precipitate in reagent trough of opened cartridge		
a)	Buffer evaporation	Excessive evaporation can lead to increased salt concentration in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for ccfDNA purification.

Ordering Information

Product	Contents	Cat. no.
QIAsymphony PAXgene Blood ccfDNA Kit (192)	For 192 preps: 2 Reagent Cartridges, Accessories and Proteinase K vials	768536
PAXgene Blood ccfDNA Tubes (100)	100 Tubes: 16 × 100 mm, 1.5 mL Additive, 10mL Blood Draw Volume	768115
QIAsymphony SP	QIAsymphony sample prep module, 1 year warranty on parts and labor	9001297
Accessories		
Cooling Adapter, EMT, v2, Qsym	Cooling adapter for EMT racks; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9020730
Cooling Adapter, 2 mL, v2, Qsym	Cooling adapter for 2 mL screw-cap tubes; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher)	9020674
Cooling Adapter, Snap-Cap Microtube QIAsymphony, Qsym	Adapter for holding Snap-Cap tubes	9020731
Sample Prep Cartridges, 8-well (336)	8-well Sample Prep Cartridges for use with the QIAsymphony SP	997002
8-Rod Covers (144)	8-Rod Covers for use with the QIAsymphony SP	997004
Filter-Tips, 1500 µL (1024)	Sterile, Disposable Filter-Tips, racked; (8 × 128). For use with the QIAsymphony SP/AS instruments	997024

Product	Contents	Cat. no.
Filter-Tips, 200 µL (1024)	Sterile, Disposable Filter-Tips, racked; (8 × 128). For use with the QIAcube and the QIAsymphony SP/AS instruments	990332
Elution Microtubes CL (24 × 96)	Nonsterile polypropylene tubes (0.85 mL maximum capacity, less than 0.7 mL storage capacity, 0.4 mL elution capacity; 2304 in racks of 96; includes cap strips)	19588
Caps for Elution Microtubes (50 × 8)	Caps for Elution Microtubes (50 × 8)	19591
Tip Disposal Bags (15)	Tip disposal bags for use with the QIAsymphony SP/AS instruments	9013395
QIAsymphony AS	QIAsymphony assay setup module, 1 year warranty on parts and labor	9001301
QIAsymphony Cabinet SP	Accessory for correct positioning of the QIAsymphony SP instruments	9020244
Related Products		
QIAamp Circulating Nucleic Acid Kit (50)	For 50 preps: QIAamp Mini Columns, Tube Extenders (20 mL), QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors and Collection Tubes (1.5 mL and 2 mL)	55114
Rotor-Gene® Q 5plex HRM® System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9001650

Product	Contents	Cat. no.
QIAcuity® Digital PCR System	Detects up to 2 fluorescent dyes, roller, USB flash memory and QIAcuity Software Suite: includes installation, training, preventive maintenance visit, and 1 year warranty on labor, travel, and parts	911002
Related products that can be ordered from BD*		
Blood Collection Set	BD Vacutainer® Safety-Lok™ 6 Blood Collection Set: 21G, 0.75 inch needle, 12 inch tubing with luer adapter; 50 per box, 200 per case	367286
BD Vacutainer One-Use Holder	Case only for 13 mm and 16 mm diameter; 1000 per case	364815

* These blood collection accessories represent typical products that can be used with PAXgene Blood ccfDNA Tubes. To find out more about these accessories, including how to order, visit **www.preanalytix.com**.

For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX kit Instructions for Use available at **www.preanalytix.com**. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
06/2016	Initial release
08/2016	Updated kit storage maximum from 6 to 12 months. Changed text in Introduction describing base technology from “silica-based” to “anion-exchange-based.” Added text to trademarks to note that all trademarks are protected by law.
02/2026	Applied formatting changes to reflect updated branding guidelines. Street address of PreAnalytiX GmbH changed from “Feldbachstrasse” to “Garstligweg 8”.

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For up-to-date licensing information and product-specific disclaimers, see the respective PreAnalytiX or QIAGEN kit handbook or user manual. PreAnalytiX and QIAGEN kit handbooks and user manuals are available at **www.preanalytix.com** and **www.qiagen.com** or can be requested from QIAGEN Technical Service or your local distributor.

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